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INTERACTION OF LIGHT AND CLOCK REGULATION IN NEUROSPORA

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Dr Genevieve M. Haddad

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13. ABSTRACT (Maximum 200 words)
Interest is focused on understanding the molecular mechanisms involved in how eukaryotic cells and organisms keep time on a daily basis and how visible light entrains the clock mechanism at the molecular level (Seminars in the Neurosciences, 7: 3-13, 1995). In the model organism, Neurospora crassa, ambient light has been shown t act either independently of, or coordinately through the circadian pacemaker. Significant advances in our understanding of how light effects a single component of the clock and thereby results in entrainment of clock phase has been made by examination of light effects on the frequency locus transcript, a known component of the clock (Cell 81, 1003 - 1012, 1995). Analysis of clock-output genes find them involved in a diverse set of cell functions, photo-inducibility to be clock independent. Promoter resection analysis shows sequences necessary and sufficient for clock-independent light regulation (Molecular and Cellular Biology, 16: 513-521 (1996) (PNAS, 93: 13096-13101 (1996). Finally, we showed progression of the clock cycle to require the transcriptional activators white-collar 1 and 2, both global regulators of Neurospora light responses, to be evolutionarily related to primitive photoreceptors and to clock associated molecules from fruit flies to mouse (Science 276, 763-769 (1997).

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Final Technical Report

United States Air Force Office of Scientific Research grant #F49620-94-1-0260

"Interaction of Light and Clock Regulation in *Neurospora*

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Jennifer Loros, Principal Investigator

United States Air Force Office of Scientific Research grant #F49620-94-1-0260, P.I. Jennifer Loros

"Interaction of Light and Clock Regulation in Neurospora"

Objectives/Project Summary:

I. What is the interplay between light and clocks at the level of gene expression?

It is now clear that there is a strong connection at the level of gene expression between photoinducibility and morning-clock regulation, and that it is broadly conserved phylogenetically: this dual regulation has been found in the fungi (e.g. the *Neurospora ccg's*), in plants (e.g. photosynthesis related genes such as CAB), and in mammalian cells (e.g. retinal transducin, and members of the immediate early gene family such as *fos* and *jun*). To date, however, little is understood about the interaction between these two levels of regulation.

--- We will examine light regulation in the *ccg-1* gene to see whether increases in transcript level driven by the clock are independent and separable from those driven by light. Is light inducibility always separable from regulation by the clock?
--- We will determine whether the level of light inducibility of the *ccg*'s is under clock-control. Does the clock modulate the light response?
--- We will examine the extent of light inducibility of our newly identified *ccg*'s, and the extent of clock regulation among previously identified light-inducible genes identified elsewhere. How tight and universal is the correlation between light inducibility and morning-clock control?

II. Identification and analysis of components in the transduction pathway that connects the clock mechanism to the photoreceptor

Despite intense interest, no single line of effort has yet identified any of the individual components involved in the light signal transduction pathway to the clock. The ways in which light effects the clock are well studied in several systems including *Neurospora*. Given the powerful genetic advantages of the *Neurospora* system, including the battery of existing mutations, it should be possible to begin to dissect the signal transduction system.

--- We will examine the clock in strains bearing mutations in the known photoresponsive elements including *wc-1, wc-2, lis-2,* and *lis-3* . Efforts will be undertaken to identify suitable zeitgebers for photoblind clocks, and to examine the characteristics of these photoblind clocks.
--- We will use a newly developed liquid culture system to select strains unable to use light to reset the clock, and to see if this clock-specific screen results in the identification of novel photo-response genes.
--- We will take advantage of the recent cloning of the *Arabidopsis* blue light photoreceptor to engage in an effort to identify the *Neurospora* blue light receptor.

III - Action of Light on a Known Clock Component

It is known that *frq* is a clock component, and we have recently shown that the level of *frq* responds to light. These data now allow us, for the first time, to directly examine the effects of light on a known component of the clock, and to begin to understand the molecular basis of clock resetting.

--- We will examine the kinetics of clock resetting and compare this with the kinetics of *frq* resetting.
--- We will determine whether the amount of resetting is altered in the known altered function clock mutants, and whether the *frq* transcript in a known loss-of-function mutant is still responsive to light.

United States Air Force Office of Scientific Research grant #F49620-94-1-0260, P.I. Jennifer Loros

"Interaction of Light and Clock Regulation in Neurospora"

STATUS OF EFFORT

Interest is focused on understanding the molecular mechanisms involved in how eukaryotic cells and organisms keep time on a daily basis and how visible light entrains the clock mechanism at the molecular level (*Seminars in the Neurosciences,* **7**:3-13, 1995). In the model organism, *Neurospora crassa*, ambient light has been shown to act either independently of, or coordinately through the circadian pacemaker. Significant advances in our understanding of how light effects a single component of the clock and thereby results in entrainment of clock phase has been made by examination of light effects on the *frequency* locus transcript, a known component of the clock (*Cell* **81**, 1003 - 1012, 1995). Analysis of clock-output genes find them involved in a diverse set of cell functions, photo-inducibility to be clock independent. Promoter resection analysis shows sequences necessary and sufficient for clock regulated gene expression are separate and distinct from regions essential for clock-independent light regulation (*Molecular and Cellular Biology,* **16**: 513-521 (1996) (*PNAS* , 93: 13096-13101 (1996). Finally, we showed progression of the clock cycle to require the transcriptional activators *white-collar 1* and *2*, both global regulators of*Neurospora* light responses, to be evolutionarily related to primitive photoreceptors and to clock associated molecules from fruit flies to mouse (*Science* **276**, 763 - 769 (1997).

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November 30, 1997

Dr. Genevieve M. Haddad
Program Manager
Directorate of Life Sciences and Environmental Sciences
110 Duncan B115
Department of the Air Force
Air Force Office of Scientific Research
Bolling Air Force Base, DC 20332-0001

Dear Dr. Haddad,

I have enclosed 6 copies of my Final Technical Report (Final Progress Report) for the United States Air Force Office of Scientific Research grant #F49620-94-1-0260 entitled "Interaction of Light and Clock Regulation in *Neurospora*". This constitutes the final report on my effort from June 1, 1994 to September 30, 1997. The on-going, long-term goals of this research are to understand, at the molecular level, the role of light in regulating the circadian clock. My co-workers and I have made significant progress during the 3 and 1/3 year course of this funding period. This has included understanding the actions of light directly on the molecular feedback loop that supports clock operation and on the regulation of the expression of genes that are additionally regulated by the clock, genes known as *ccg*'s or clock-controlled genes. The approach is based on experiments that have established the products of the *frq* (*frequency*) locus as components of the clock in the model system *Neurospora crassa*. We have shown that light- induced increases in the level of transcript arising from *frq* is the basis of light resetting of the clock in *Neurospora*, and predicted that such a light-induced increase in the rate of transcription will be the basis for light-induced resetting of day-phase clocks such as those found in mammals including people. We are substantiating our predictions with a recent collaboration that has resulting in the submission of a paper describing the transcriptional activation of the mouse per gene, *mper*, by light. Additionally, as we extended our understanding of light regulation of the Neurospora *frq* gene, a known clock component we showed that two genes, *wc-1* and *wc-2* (*white-collar 1&2*), both global regulators of light responses in *Neurospora*, are also, surprisingly, required for progression of the clock cycle in the dark. These data were a demonstration at the molecular level of the extent to which the light input pathway to the clock, which can require both *wc*'s, is merged with the clock cycle itself. In additional to the general importance of this information for understanding how clocks are reset by light, the specific importance of understanding these genes is attested to by the finding that the mouse clock-associated gene *clk* (*clock*) has sequence and probable functional homologies to the *wc* genes.

Specifically, the clock in *Neurospora* is comprised of a negative feedback loop wherein the *frq* gene encodes the FRQ protein which, in turn, feeds back to turn off the gene. In work supported by this effort we have shown that light delivered at any point

within the circadian cycle acts rapidly to increase the level of *frq* transcript by stimulating transcription of *frq* . The magnitude of the light-induced increase in *frq* mRNA and the extent of clock resetting are correlated, with a threshold for each response of 8 mmoles photons/m^2/s. This threshold, along with the kinetics and magnitude of this response are consistent with a model in which elevation of the level of *frq* transcript in the cell is the initial clock-specific event involved in resetting of the clock by light. We have also begun to dissect the signal transduction system for light-resetting of the clock by examination of photoregulation of *frq* in two photo-blind mutant strains, *wc-1* and *wc-2*. The immediate and transient light-induced accumulation of *frq* is blocked in *wc-1* but not in *wc-2* . We also examined clock entrainment and function in these strains following light to dark steps and by non-photic temperature cues and found both strains are unable to support circadian rhythmicity. The *wc1* and *wc-2* genes, both required for all non-clock photoresponses in *Neurospora*, encode DNA binding proteins that contain PAS domains and are believed to act as transcriptional activators. Data show *white collar-1 (wc-1)* to be absolutely required for light-induced accumulation of *frequency* transcript and for robust cycling of *frq* in the dark. The *wc-2* gene, which also has a role in the sustained response of *frq* to light, is necessary for proper clock function. Thus, the WC proteins appear to correspond to the positive elements in the feedback loop that have been predicted based on theoretical considerations. The PAS domains in these proteins link them at the sequence level to both the *period* gene from insects and the *clk* gene from mouse, suggesting evolutionary conservation of proteins involved in clock function. In addition to their identification in clock-associated molecules, PAS domains are found in molecules involved in photoreception and in signal transduction. The presence of PAS in the *white-collar* proteins - involved in both signal transduction and in clock function - suggest that photoreception and circadian rhythmicity are linked not only at the physiological level but also at the molecular level, and suggests that circadian oscillators may have evolved from ancient proteins involved in signal transduction and photoresponsivity.

We have also made significant progress in understanding the interactions between light and the clock in regulating gene expression in our model system. Clock controlled genes display a variety of biochemical functions and respond to diverse factors influencing the cell. There are now 8 canonical *ccg*'s and 3 additional clock-controlled genes identified by other labs working on light and developmentally-regulated genes, yielding a total of 11 genes in *Neurospora* clearly involved with clock regulated processes and not a part of the oscillator itself. Not all of the *ccg*'s are associated with light or development; one encodes glyceraldehyde-3-phosphate-dehydrogenase, a key glycolytic control enzyme that is not light, stress or developmentally regulated. The functions of other *ccg*'s may be associated with stress or development. At the level of gene expression *ccg*'s show complex regulation, responding to several environmental stimuli in addition to endogenous clock control. The tight association between clock regulation and light regulation found for *ccg*'s in the past has now been separated at the level of the promoter sequence (regions responsible for clock regulation can be separate from regions responsible for light regulation as in the *ccg*-2 promoter) as well as at the gene level. Three of the *ccg*'s show no response to light at the level of gene expression.

Dissection of model systems at the molecular and biochemical level establishes paradigms and therefore provides useful approaches for understanding more complex systems. Recent data from our lab and others suggests evolutionary conservation from fungi to mammals of the basic molecules involved in circadian timing. Additionally, an important outcome from our work in Neurospora is that all clocks may turn the simple, unidirectional signal of a light pulse into a bi-directional (phase advance or delay) signal,

simply by changing the position of the peak of a clock molecular component (mRNA or protein) rhythm in relation to the time of day. The further prediction, that light activated transcription of a clock component is the method employed by day active organisms including mammals, is currently under investigation, with strong supporting results.

If there is additional information with which I can provide you, please do not hesitate to call.

Sincerely,

Jennifer J. Loros
Associate Prof. of Biochemistry

United States Air Force Office of Scientific Research grant #F49620-94-1-0260, P.I. Jennifer Loros

"Interaction of Light and Clock Regulation in Neurospora"

PERSONNEL SUPPORTED by the research effort:

Dr. Jennifer Loros, P.I.
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Dr. Deborah Bell Pedersen, Research Associate
Dr. Norman Garceau, Research Associate
Dr. Hyseon Cho, Research Associate
Ms. Chenghua Luo, Graduate Student
Ms. Anne Cole, Graduate Student
Dr. Michael Collett, Research Associate
Dr. Deanna Denault, Research Associate
Dr. Kwangwon Lee, Research Associate
Ms. Julia Doster, Undergraduate Student, Presidential Scholar, Dartmouth College, Senior Honor's Thesis Student
Ms. Melissa Lodoen, Undergraduate Student, Dartmouth College, Senior Honor's Thesis Candidate

COLLABORATIVE PERSONNEL associated with the research effort:

Dr. Jay Dunlap, Professor, Dartmouth Medical School
Dr. Guiseppe Macino, Professor, University of Rome
Dr. Stephen Free, Professor, State University of New York
Dr. Hitoshi Okamura, Professor, Kobe University School of Medicine

PUBLICATIONS listing AFOSR #F49620-94-1-0260 for support

PEER REVIEWED PUBLICATIONS

Arpaia, G. **J.J. Loros**, J. C. Dunlap, G. Morelli, and G. Macino, Light Induction of the Clock-Controlled Gene ccg-1 is not Transduced Through the Circadian Clock, *Molecular General Genetics*, **247**:157-163, 1995

Crosthwaite, S., **Loros, J. J.** and Jay C. Dunlap, Light-Induced Resetting of a Circadian Clock is Mediated by a Rapid Increase in *frequency* Transcript. *Cell* **81**, 1003 - 1012 (1995) **(cover article)**

Bell-Pedersen, D., J. C. Dunlap and **J. J. Loros**, Distinct cis-Acting Elements Mediate Clock, Light and Developmental Regulation of the *Neurospora crassa eas* (ccg-2) Gene. *Molecular and Cellular Biology*, 16: 513-521 (1996)

Bell-Pedersen, D., Shinohara, M., **Loros, J. J.** and J. C. Dunlap. Circadian clock-controlled genes isolated from *Neurospora crassa* are late night- to early morning-specific. *PNAS*, 93: 13096-13101 (1996)

Garceau, N., Liu, Y. **Loros, J. J.** and J. C. Dunlap. Alternative initiation and time-specific phosphorylation reflect complex regulation of the circadian clock protein FREQUENCY. Cell 89, 469 - 476 (1997)

Liu, Y., Garceau, N., **Loros, J. J.** and J. C. Dunlap. Thermally regulated translational control mediates aspects circadian temperature responses in the Neurospora circadian clock. Cell **89**, 477 - 486 (1997)

Crosthwaite, S. K., Dunlap, J. C. and **J. J. Loros**. Neurospora *wc-1* and *wc-2*: Transcription, Photoresponses, and the Origins of Circadian Rhythmicity. Science **276**, 763 - 769 (1997)

Shigeyoshi, Y., Taguchi, K., Yamamoto, S., Takekida,S., Yan, L., Tei, H., Moriya, T., Shibata, S., **Loros, J.J.**, Dunlap, J. C., and H. Okamura. Light-Induced Resetting of a Mammalian Circadian Clock is Associated with Rapid Induction of the *mPer*1 Transcript. Cell *in press* (1997)

INVITED REVIEWS

Jennifer J. Loros. The Molecular Basis of the *Neurospora* Clock. *Seminars in the Neurosciences*. **7** (1) pp.3-13 (1995)

Jay C. Dunlap, **Jennifer J. Loros**, Martha Merrow, Susan Crosthwaite, Deborah Bell-Pedersen, Norman Garceau, Mari Shinohara, Hyeseon Cho, and Chenghua Luo. The Genetic and Molecular Dissection of a Prototypic Circadian System. *Progress in Brain Research* (Elsevier), 111:11-27
(1996)

Jennifer J. Loros, Jay C. Dunlap, Susan Crosthwaite, Deborah Bell-Pedersen, Norman Garceau, Mari Shinohara, Hyeseon Cho, and Chenghua Luo, Yi Liu, Michael Collett, Anne Cole, Christian Heintzen and Martha Merrow. Light, Light Responsive Genes, and the Mechanism of the Circadian Clock in *Neurospora.* Proceedings of the European Photobiology Federation, Meeting 9/97 Vienna, *in press* 1997

Bell-Pedersen, D., N. Y. Garceau, and **J.J. Loros**. Circadian Rhythms in Fungi. J. Genet. 1996 75(3): 387-401 (1996)

Dunlap, Jay C. and **Jennifer J. Loros**. Molecular and Genetic Analysis of Circadian Rhythms. invited review for *Microbiological Reviews*. to be completed in 1998

J.J. Loros Light and the circadian clock in Neurospora. ASM News, invited review, for January 1998

BOOK CHAPTER

Jennifer J. Loros and Jay C. Dunlap. 1997. Molecular Genetics of Circadian Rhythms in *Neurospora*, in *Handbook of Behavioral Neurobiology*, ed. Takahashi, J. Turek, F.W. and Moore, R.Y. Plenum Press, New York. in press 1997

INTERACTIONS & TRANSITIONS June 1994 to December, 1997, including invitations for 1998 arising from AFOSR sponsored work:

MEETINGS AND SYMPOSIA ORGANIZATION

1997 Chair of Neurospora Policy Committee, led Policy discussions, Business Meeting during
Neurospora Workshop, committee organized full day session, 19th Fungal Genetics Conference, Asilomar, CA

1995 American Physiological Society Conference on Understanding the Biological Clock: From Genetics to Physiology, Conference Organizer, Dartmouth, Hanover, New Hampshire

1995 Chronobiology Gordon Conference, session chair, Barga, Italy

INVITED LECTURES AND MEETINGS

1998 University of Georgia, Graduate Student Invitation
1998 FASEB Neurobiology of Vertebrate Entrainment meeting, Snowmass, CO
1998 Boston University, Boston, MA
1998 Brandeis University, Worcester, MA
1998 University of Massachusetts, Amherst, MA
1998 University of British Columbia, Vancouver, Canada
1997 AFOSR, Chronobiology and Neural Adaptation Program Review, Colorado Srings, CO
1997 Gordon Conference on Chronobiology, speaker, New London, NH
1997 York University, Graduate Student Symposium speaker
1997 American Society for Microbiology, Miami, co-convenor &speaker in symposium
1997 Fungal Genetics Conference, Pacific Grove, CA
1997 EMBO Workshop, Molecular Mycology, Vienna, Austria
1996 12th International Congress on Photobiology, Vienna, Austria
1996 INSERM, Circadian Light Reception and Regulation, Lyon, France
1996 Society for Research on Biological Rhythms, evening commentary speaker on days session, session chair, Amelia Island, Florida
1996 Dartmouth Medical School, Hanover, New Hampshire
1996 Tulane Medical Center, New Orleans, LN
1995 Worcester Foundation for Biomedical Research, Shrewsbury, MA
1995 Gordon Conference on Chronobiology, session chair, speaker, Barga, Italy
1995 American Physiological Society, Understanding the Biological Clock, speaker, Hanover, New Hampshire
1995 Distinguished Lecturer, Brandeis Scientific Outreach Program, Public Lecture on Biological Timing, Waltham, MA
1994 Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT
1994 2nd Life Sciences Symposium, Dartmouth, NH
1994 NSF Center for Biological Timing U. S.-Japan Joint Conference, Hawaii

CONSULTATIVE AND ADVISORY FUNCTIONS

United States Air Force Office of Scientific Research -

Scientific Program Attendee for the Chronobiology and Neural Adaptation Program Review, September 18-20, 1997, including submitting brief meeting report to Dr Haddad

National Science Foundation

- Ad Hoc reviewer for several Programs including Eukaryotic Genetics, Biochemical Approaches to Genetic Systems, Developmental Biology, Microbial Genetics, Behavioral Neuroscience and Metabolic Biochemistry.

National Institute of Health,

Molecular, Cellular & Developmental Neurobiology Study Section 1994
Chair, Molecular, Cellular & Developmental Neurobiology Special Emphasis Study Section 1996

Neurospora Policy Committee,

1993-1997, elected to 4 yr. term on governing board of international policy committee overseeing policy and the Fungal Genetics Stock Center, 1995-1997 elected Chair

TRANSITIONS

A 1997 written report for an engineering consulting firm, Nottingham Spirk, detailing the known regulatory effects of light and circadian rhythms on the production of mycotoxin bearing conidiospores from fungi found as common contaminants in foodstuffs.

Dr. Paul Brokaw
Nottingham Spirk
11310 Juniper Dr.
Cleveland, OH 44106
FAX: 216 231-6275
phone # 216-231-7830

HONORS AND AWARDS

Aschoff's Rule, May 1996 awarded at the Society for Research on Biological Rhythms Meeting, Florida, awarded yearly to a researcher in biological rhythms.

Appointed Associate Editor of the journal GENETICS

Current candidate, National Science Foundtion Creativity Award

NEW DISCOVERIES, INVENTIONS AND PATENT DISCLOSURES

None